INTERNATIONAL RESEARCH JOURNAL ON ADVANCED SCIENCE HUB
e-ISSN : 2582 - 4376
Open Access
RSP SCIENCE HUB
(The Hub of Research Ideas)
Available online at www.rspsciencehub.com

Special Issue of Second International Conference on Advances in Science Hub (ICASH 2021)

Isolation and Identification of *Bacillus* Strains for Bioconcrete

Ch. Jyothi[1], M.A. Singara Charya[2]

[1]Research Scholar, Department of Microbiology, Kakatiya University, Warangal, Telangana, India.

[2]Professor, Department of Microbiology, Kakatiya University, Warangal, Telangana, India.

chittampallyjyothi@gmail.com[1]

Abstract

Crack formation is a very common phenomenon in concrete structures which allows the water and chemicals through the cracks and decreases the durability and strength. For repairing the cracks developed in the concrete, it requires maintenance and special type of treatment. So, to overcome this problem an autonomous self healing mechanism is introduced into the concrete which helps to repair the cracks with the help of Bacillus bacteria by producing calcium carbonate crystals which seals the micro cracks and pores in the concrete. For the isolation of bacteria nearly eighty soil samples were collected from soils of extreme environments and cultured in three different media. A total of two thousand seventy colonies were observed and fifty five Bacillus colonies were isolated and identified by Gram staining and different biochemical tests.

Key words: Self healing, Bacillus, concrete, extremophilic, precipitation.

1. Introduction

Concrete is the most commonly used construction material, but most of the structures are prone to cracking with time and different reasons such as material limitations, design gaps, construction practices as well as exposure conditions to the environment. Cracking is a common phenomenon in concrete due to the relatively low tensile strength [1]. Cracking in the surface layer of concrete mainly reduces its durability and can lead to damage of the mineral matrix and corrosion of steel. Because of these disadvantages an alternative technique for the improvement of the durability of concrete which biologically produces calcium carbonate crystals to seal the cracks on the surface of the concrete by using different types bacterial strains was introduced. Though concrete is quite strong mechanically, it suffers from several drawbacks, such as low tensile strength, permeability to liquid and consequent corrosion of reinforcement, susceptibility to chemical attack and low durability [2]. Self healing concrete biologically produces calcium carbonate crystals to seal the cracks which appear on the surface of the concrete [3]. In this process selected types of genus *Bacillus* along with calcium based nutrient in the presence of oxygen and the soluble calcium is converted to insoluble calcium carbonate by ureolytic activity [4]. When cracks appears on the concrete structure water starts to seep through spores of the bacteria which enable to start microbial activities after contact with the water and oxygen. The soluble nutrients are converted to insoluble calcium carbonate which solidifies on the cracked surface thereby sealing it up [5]. It impersonate the process of bone healing of fractures in the human body are naturally restored by osteoblast cells that mineralize to reform the bones [6-9] and bacterial based self healing agent is believed to remain hibernated within the concrete up to 200 years [10],[11].This paper mainly reports the investigation of microorganisms isolated from extreme environments and are known to survive in alkaline environments, some are able to produce urease enzyme which involve in microbial induced calcite precipitation (MICP) which is also called as bio-mineralization. MICP is a natural phenomenon associated with a wide

range of bacterial species in an alkaline environment rich in Ca+ [12]. The addition of urea to the microorganism allows the conversion of urea to dissolved inorganic carbon and ammonium, subsequently releasing the ammonium to the environment [13]. This biomineralogy concept leads to the potential invention of a new material i.e, Bacterial concrete, an inherent and self repairing biomaterial that can remediate the cracks and fissures in concrete [3]. The mechanism of microbial calcium carbonate precipitation occurs worldwide in natural systems such as, oceans, microbial mats, biofilms and stromatolites[14-17],especially in oceans[18],[3].

2. Material and methods

In order to identify right bacteria for the development of bioconcrete it is necessary to isolate urease producing bacteria for the production of calcium carbonate which was used for the development of bioconcrete.

2.1 Isolation of bacterial colonies from soil samples

Soil samples were collected from brick kilns, furnaces, rocks of different areas of Warangal district i.e, in Hanamkonda, Bheemaram village Bairanpally, Gopalpur, Yerragollahpahad, Marigadi, Chowdaram and in Khammam Dist (Maddhulapally village) and marine samples from Vishakapatnam are cultured in nutrient agar media and Zobell medium by pour plate method and incubated for 24 hrs at 37^0 C. The isolated bacterial cultures were separated and sub cultured several times to obtain pure cultures.

2.2 Morphological, biochemical studies of bacterial isolates

Bacterial colonies obtained by pure culture technique are subjected for identification tests such as Gram staining, Endospore staining and biochemical tests such as catalase, oxidase, starch hydrolysis, eosin methylene blue, macconkey media, indole production, urease test etc for the identification of *Bacillus* species. To characterize all the bacterial colonies according to conventional, physiological and biochemical characterization tests were carried out as described in Bergey's Manual of Systemic Bacteriology [19].

3. Results & discussion

3.1 Isolation of Bacterial colonies from soil samples

Eighty soil samples were collected and cultured in different media for the growth of bacteria, approximately two thousand seventy bacterial colonies were observed and fifty five *bacillus* colonies were isolated and were used for screening of potent strains.

3.2 Screening and biochemical studies of bacterial isolates

Fifty five bacterial strains obtained by pure culture technique are subjected for identification tests such as Gram staining, Endospore staining and biochemical tests such as catalase, oxidase, motility test, starch hydrolysis, simmon's citrate agar, macconkey media, gas production, indole production, Urease test etc for the identification of *Bacillus* species. The biochemical test results for the fifty five bacterial strains obtained were reported in the following table:

Table.1. showing Biochemical test results for the isolated bacterial strains

SAMPLE	C.T	U.T	SCA	M.T	I.P	O.T	S.H	G.P	MAC	G.S	S.F
CJ-1	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	CL	-ve	-ve
CJ-4	-ve	w+ve	-ve	+ve	-ve	+ve	+ve	+ve	LPC	+ve	-ve
CJ-7	-ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	CL	-ve	-ve
CJ-8(w)	-ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	LPC	-ve	-ve
CJ-8(y)	+ve	w+ve	-ve	-ve	+ve	+ve	-ve	+ve	CL	-ve	-ve
CJ-9	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	LPC	+ve	+ve
CJ-10	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	CL	-ve	-ve
CJ-11(Y)	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	CL	+ve	-ve
CJ-11(b)	-ve	w+ve	-ve	+ve	-ve	+ve	+ve	+ve	LPC	+ve	-ve
CJ-12	-ve	w+ve	+ve	-ve	-ve	+ve	-ve	+ve	LPC	+ve	+ve
CJ-16	-ve	w+ve	-ve	+ve	+ve	-ve	-ve	-ve	LPC	+ve	+ve
CJ-17	-ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	CL	-ve	-ve

CJ-20	+ve	w+ve	-ve	+ve	-ve	-ve	+ve	-ve	CL	-ve	-ve
CJ-21	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	LPC	+ve	+ve
CJ-24	-ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	CL	-ve	-ve
CJ-26	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	LPC	+ve	+ve
CJ-28	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	LPC	+ve	+ve
CJ-31(B)	+ve	w+ve	-ve	-ve	+ve	+ve	-ve	-ve	CL	-ve	-ve
CJ-31(C)	-ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	CL	-ve	-ve
CJ-32	+ve	w+ve	+ve	-ve	+ve	+ve	+ve	+ve	LPC	-ve	-ve
CJ-36	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	LPC	+ve	-ve
CJ-41(B)	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	CL	+ve	-ve
CJ-41(Y)	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	CL	-ve	-ve
CJ-41(w)	-ve	w+ve	-ve	-ve	+ve	+ve	+ve	+ve	LPC	+ve	+ve
CJ-43	-ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve	LPC	-ve	-ve
CJ-46	+ve	w+ve	-ve	-ve	+ve	-ve	-ve	-ve	CL	-ve	-ve
CJ-47(B)	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	CL	-ve	-ve
CJ-47(Y)	-ve	w+ve	+ve	+ve	-ve	+ve	-ve	-ve	CL	-ve	-ve
CJ-51	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	LPC	+ve	-ve
CJ-52	+ve	w+ve	-ve	-ve	+ve	+ve	+ve	-ve	LPC	+ve	+ve
CJ-55	-ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	LPC	+ve	-ve
CJ-56(B)	+ve	w+ve	+ve	-ve	-ve	-ve	+ve	+ve	CL	+ve	-ve
CJ-56(w)	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	LPC	+ve	-ve
CJ-59(y)	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	CL	-ve	-ve
CJ-59(B)	+ve	w+ve	+ve	+ve	-ve	-ve	-ve	+ve	CL	-ve	-ve
CJ-60	+ve	w+ve	+ve	+ve	-ve	+ve	+ve	+ve	LPC	+ve	+ve
CJ-62	+ve	w+ve	-ve	-ve	-ve	-ve	+ve	-ve	CL	-ve	-ve
CJ-65(w)	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	CL	-ve	-ve
CJ-65(B)	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	CL	-ve	-ve
CJ-66	-ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	CL	-ve	-ve
CJ-67(Y)	-ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	LPC	-ve	-ve
CJ-67(w)	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	LPC	-ve	-ve
CJ-67(B)	-ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	CL	-ve	-ve
CJ-67(J)	-ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	LPC	+ve	-ve
CJ-68(w)	+ve	w+ve	-ve	-ve	+ve	-ve	+ve	-ve	LPC	+ve	+ve
CJ-68LB	+ve	w+ve	+ve	+ve	-ve	+ve	+ve	+ve	LPC	+ve	-ve
CJ-70	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	CL	-ve	-ve
CJ-71(B)	+ve	w+ve	+ve	+ve	-ve	+ve	+ve	-ve	LPC	+ve	-ve
CJ-71(Y)	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	CL	-ve	-ve
CJ-72	+ve	w+ve	-ve	-ve	+ve	-ve	+ve	-ve	LPC	+ve	+ve
CJ-75(B)	-ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve	CL	-ve	-ve
CJ-75(w)	+ve	w+ve	+ve	+ve	-ve	+ve	+ve	+ve	LPC	+ve	+ve
CJ-78	+ve	w+ve	-ve	+ve	-ve	+ve	+ve	+ve	LPC	+ve	+ve
CJ-79(Y)	+ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	CL	-ve	-ve
CJ-79(J)	+ve	w+ve	-ve	-ve	+ve	-ve	+ve	-ve	LPC	+ve	+ve

Abbreviations: -ve: Negative, +ve: Positive, w+ve: Weak positive, CL: Colour less, LPC: Light Pink Colonies, CT: Catalasse Test, UT: Urease Test, SCA: Simmon Citrate Agar, MT: Motility Test, IP: Indole production test, OT: Oxidase test, SH: Starch hydrolysis, MAC: Mac Conkey agar media, GS: Gram staining, SF: Spore formation

From the biochemical tests it was observed that 25 isolates showed urease positive, 23 isolates are weak positive and 7 isolates showed negative results for urease test. To check the efficiency of

the bacterial samples for calcium carbonate precipitation (which showed positive results for urease activity) cultures were grown on calcium carbonate precipitating media. Out of which 13 samples were more precipitated within a short period of time i.e, results were noted for every 24 hrs and compared to that of all incubated cultures. Samples which showed better results in calcium carbonate precipitation media were further used for quantitative analysis and after that selected bacterial samples were studied for morphological, molecular identification upto the species level. Among the 13 isolates, four samples were selected based on their better precipitation in calcium carbonate precipitating media and were identified as *Bacillus* species by biochemical tests. Molecular identification of these four isolates were performed by amplification and sequencing the 16S rRNA gene and confirmed as *Bacillus* species which was done by National Chemical Laboratory (Pune), the sequences thus obtained was submitted in National Centre for Biotechnology Information (NCBI GenBank Accession Numbers – MN809595, MN849173, MN849426 and MN849881). The selected four isolates CJ-9 , CJ-21 , CJ-26, CJ-28 were found to be more efficient and belongs to *Bacillus,* based on nucleotide homology and phylogenetic analysis the strain CJ-9 is identified as (*Bacillus thuringenisis*), CJ-21 is (*Bacillus albus*), CJ-26 is (*Bacillus mycoides*) and CJ-28 is (*Bacillus anthracis*). The isolated bacteria which were obtained are screened quantitatively by gram staining, endospore staining and for the urease test. Urease produced by bacteria is widely known to precipitate calcium carbonate, one of the main components of concrete, which is referred as microbial concrete enzyme. To remediate building materials urease needs to be active and stable in alkaline environment (pH 9-11) that also include high temperature [6]. The screening for urease producing bacteria was conducted by using urease agar medium which changes the colour from pale yellow to pink indicates positive urease activity. Several studies have reported that urease agar base can be used as a quick method for primary screening of urease producing bacteria which is suitable for biocementation purposes [20-22]**.** Urease agar base contains urea and phenol red which acts as a pH indicator. When urea is hydrolysed by the bacteria, ammonia is released and becomes accumulated in the medium which increases the pH of the environment making it alkaline [23].All the isolated bacteria of the present study were identified as *Bacillus* and most of the calcifying bacteria belong to the *Bacillus* genera. The genus *Bacillus* has been mostly used for the biological development of calcium carbonate based minerals as, which is considered as a ureolytic bacteria. The formation of calcium carbonate by using this type of bacteria is because of the hydrolysis of urea to carbondioxide and ammonia which increases the pH of the medium at the cell surface and promotes formation of calcium carbonate crystals **[**24], [25], [6]**.** Similar results were reported by Hammed *et al,* [23] for the formation of calcium carbonate by *sporosarcina pasterii.* Numerous studies have mainly adopted the use of *Sporosarcina pasteurii* as their preferred ureolytic bacteria for MICP process because it is non-pathogenic and has quick capability to produce urease [26-32]. All the bacterial isolates selected for the present study were capable of forming endospores. Endospores are special resistant dormant, tough, and non-reproductive structures produced by some bacteria within the cell in the phylum Firmicutes [33], [34]**.** Endospores are extremely resistant to heat, chemicals, radiation, desiccation, enzymatic destruction and are capable of surviving in hostile environmental conditions and they can germinate into a vegetative cell within 90 min [35]. By the formation of endospores, bacteria can withstand large mechanical and chemically induced stresses during concrete mixing [36]. Ercole *et al,* [37] studied the bacteria for calcification and used *Bacillus* species for the development of bioconcrete.

Conclusion

From the results it was evident that from the isolation and identification studies, the selected four isolates CJ-9, CJ-21, CJ-26, CJ-28 in the present study were found to be more efficient and belongs to *Bacillus,* based on nucleotide homology and phylogenetic analysis the strain CJ-9 is identified as (*Bacillus thuringenisis*), CJ-21 is (*Bacillus albus*), CJ-26 is (*Bacillus mycoides*) and CJ-28 is (*Bacillus anthracis*) and they were investigated and used for further studies in concrete application.

Acknowledgements

I would like to thank Head, Department of Microbiology, Kakatiya University, Warangal for providing laboratory facilities and I (CH.JYOTHI) greatly thankful to RGNF, UGC – New Delhi, for providing financial assistance.

References

[1]. Chahal, N. Siddique, R. and Rajor, A. (2012). Influence of bacteria on the compressive strength, water absorption and rapid chloride permeability of fly ash concrete. Journal of Construction and Building Materials. 28.351-356.

[2].Mehta, P. K. (1999). Factors influencing durability of concrete structures, Advancement in concrete technology, International Concrete Journal, 21, 69-75.

[3].Ramchandran, S.K. Ramakrishnan, V and Bang, S. S. (2001). Remediation of concrete using microorganisms, ACI Materials Journal, 98 (1) 3-9.

[4].Wu, M. Johannesson, B and Geiker, M. (2012). Self Healing in cementitious materials and engineered cementitious composite as a self healing material. Journal of Construction and building Materials. 28, 571-583.

[5].Depaa, R.A.B and Felix Kala, T. (2015). Experimental Investigation of Self Healing Behavior of Concrete using Silica Fume and GGBFS as Mineral Admixtures. Indian Journal of Science and Technology, 8: 0.17485/ijst/2015/v8i36/87644.

[6].Stocks Fischer, S. Galinath, J. K. Bang, S. S. (1999). Microbiological precipitation of $CaCO_3$. Journal of Soil Biology and Biochemistry. 31, 1563-1571.

[7].Jonkers, H.M. (2007). Self healing concrete: A biological approach in self healing materials - An alternative approach to 20 centuries of material science (ed.S.Van der Zwagg), Springer, The Netherlands, 195-204.

[8].Sangadji, S. and Schlangen, E. (2012). Self Healing of Concrete Structures - Novel Approach Using Porous Network Concrete. Journal of Advanced Concrete Technology. 10, 185–194.

[9].Souradeep, G. and Kua, H.W. (2016). Encapsulation Technology and Techniques in Self Healing Concrete. Journal of Materials on Civil Engeenering. 28,161-165.

[10].H.G.Schlegel, "General Microbiology", 7th Edition , Cambridge University Press,(1993).

[11].Meldrum, F.C. (2003). Calcium carbonate in biomineralisation and biomimitic chemistry, International Materials Reviews, 83, 187-224.

[12].Achal, V. and Pan, X. (2011). Characterization of urease and carbonic anhydrase producing bacteria and their role in calcite precipitation. Journal of Current Microbiology. 62, 894-902.

[13].De muynck, W. De Belie, N. Verstrete, W. (2010). Microbial carbonate precipitation in construction materials - A review. Ecological Engineering, 36, 118-136.

[14].Krumbein, W.E. Cohen, Y. Shilo, M. (1977). Solar Lake (Sanai) Stromatolitic cyanobacterial mats. Journal of Limnol Oceanogr. 22, 635-656.

[15].Riding, R. (2000). Microbial carbonates :The geological record of calcified bacterial –algal mats and biofilms. Journal of Sedimentology. 47, 179-214.

[16].Arp, G. Reimer, A. and Reitner, J. (2001). Photosynthesis-induced biofilm calcification and calcium concentrations in Phanerozoic oceans. Journal of BioScience. 292,1701-1704.

[17].Ludwig, R. Al –Haroni, F.A. DeBeer, D.and Jonkers, H.M. (2005). Photosynthesis-controlled calcification in a hypersaline microbial mat. Journal of Limnol Oceanogr. 50,1836-1843.

[18].M. R. Leeder (1982) Sedimentology, George Allen and Unwin (publishers) Ltd, London, pp 344.

[19].J.G. Holt, "Bergey's Manual of Systemic bacteriology", Second Ed, Springer,(2001).

[20].Burbank, M.B. Weaver, T. J. Williams, B.C. and Crawford, R.L. (2012). Urease activity of ureolytic bacteria isolated from six soils in which calcite was precipitated by indigenous bacteria. Journal of Geomicrobiology. 29, 389-395.

[21].Chahal, N. Rajor, A. and Siddique, R. (2011). Calcium carbonate precipitation by different bacterial strains. African Journal of Biotechnolog. 10, 8359-8372.

[22].Hammes, F. Boon, N. De Villiers, J. Verstraete, W. and Siciliano, S.D. (2003). Strain-specific ureolytic microbial calcium carbonate precipitation. Journal of Applied Environmental Microbiology. 69, 4901-4909.

[23].Hammad, I.A. Talkhan, F.N. and Zoheir, A.E. (2013). Urease activity and induction of calcium carbonate precipitation by *Sporosarcina pasteurii* NCIMB 8841. Journal of Applied Sciences Research. 9, 1525-1533.

[24].Castanier,S. Le Metayer-Levrel, G. and Perthuisot, J.P. (1999). Co-carbonates precipitation and lime stone genesis – the microbiologist point of view. Journal of sedimentary geology. 126, 9-23.

[25].Tiano, P. Biagiotti, L. and Mastromei, G. (1999). Bacterial bio mediated calcite precipitation for monumental stones conservation: methods and evaluation. Journal of Microbiological methods. 36, 139-145.

[26].Achal, V. Mukherjee, A. Basu, P.C. and Reddy, M. S. (2009). Strain improvement of *Sporosarcina pasteurii* for enhanced urease and calcite production. Journal of Industrial Microbiology and Biotechnology. 36, 981-988.

[27].Al-Thawadi, S.M. (2008). High strength in-situ biocementation of soil by calcite precipitating locally isolated ureolytic bacteria. (Doctoral thesis), Murdoch University, Perth, Australia.

[28].Cheng, L. and Cord-Ruwisch, R. (2013). Selective enrichment and production of highly urease active bacteria by non-sterile (open) chemostat culture. Journal of Industrial Microbiology and Biotechnology. 40, 1095-1099.

[29].Cuzman, O.A. Rescic, S. Richter, K. Wittig, L. and Tiano, P.(2015b). *Sporosarcina pasteurii* use in extreme alkaline conditions for recycling solid industrial wastes. Journal of Biotechnology. 214, 49-56.

[30].Kang, C.H. Choi, J.H. Noh, J. Kwak, D.Y. Han, S.H. and So, J.S. (2014). Microbially induced calcite precipitation-based sequestration of strontium by *Sporosarcina pasteurii* WJ-2. Journal of Applied Biochemistry and Biotechnology. 174, 2482-2491

[31].Wei, S. Cui, H. Jiang, Z. Liu, H. He, H. and Fang, N. (2015). Biomineralization processes of calcite induced by bacteria isolated from marine sediments. Brazilian Journal of Microbiology. 46, 455-464.

[32].Whiffin, V.S. (2004). Microbial $CaCO_3$ precipitation for the production of biocement. (Doctoral thesis), Murdoch University, Perth, Australia.

[33].Murray, M. Patrick, R. Ellen Jo Baron (2003) Manual of Clinical Microbiology. **1.** Washington, D.C:ASM.

[34].Michael Hogan, C. (2010). *"Bacteria"*. In Sidney Draggan; C.J. Cleveland (eds.). Encyclopedia of Earth. Washington DC: National Council for Science and the Environment. Archived from *the original on 2*011-05-11.

[35].S. Geeta and R.S. Mehrotra, "Principles of microbiology". 1st edition, New Delhi, "Tata McCraw Hill Education Private Limited", (2009*).*

[36].Saripanti, J.L. and Bonifacino. A. (1996). Comparative sporocidal effects of liquid chemical agents. Journal of Applied Environmental Microbiology. 62(2), 545-551.

[37]. Ercole, C. Cacchio, P. Botta, A. Centiv, L. Lepidi, A. (2007). Bacterially induced mineralization of calcium carbonate:the role of exopolysaccharides and capsular polysaccaharides. Microscopy and micro analysis, 13, 42-50.